Quantitative Approaches for Using Color Infrared Photography for Assessing In-Season Nitrogen Status in Winter Wheat

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ABSTRACT

Due to the timing and rates of N applications in wheat (*Triticum aestivum* L.), the potential exists for high N loading to the environment. Plant tissue tests offer growers the ability to determine in-season N status, and to optimize N applications and N use efficiency. However, sampling and N analysis can be costly, difficult, and time consuming. Remote sensing may offer a solution to these problems. The objectives of this study were to determine (i) if remote sensing could be used to estimate in-season N status, (ii) if within-field calibration would improve the ability of remote sensing to estimate crop N status, and (iii) if optimum N rates could be estimated using remote sensing. Research was conducted in 1999 to 2001 at eight sites. Two sites had randomized complete block designs with variety, seeding rate, and N rate as treatments. Six sites had a single seeding rate and wheat variety. Biomass was found to influence spectral measurements of in-season N status. A strong relationship between the normalized difference vegetation index (NDVI) and growth stage (GS)-30 whole-plant N concentration (R^2 = 0.69) and GS-30 N uptake (R^2 = 0.61) was found. Within-field calibration did not improve the estimation of in-season N status by NDVI. While it was possible to use NDVI to estimate GS-30 N uptake, predicted N fertilizer rates based on N uptake were highly unreliable. However, NDVI reliably predicted GS-30 N fertilizer rates based on whole-plant N concentration for wheat that had mean GS-30 biomass values >1000 kg ha^{-1}.

Efficient use of N fertilizer is important for the economic sustainability of soft red winter wheat production and to abide by environmental regulations designed to limit nonpoint-source pollution from agriculture. Due to the timing and rates of N applications to wheat, the potential exists for high loading of N into the environment. This is especially true in the humid southeastern USA where sandy soils and high rainfall during the fall and winter can leach or dentitrify N applied before wheat planting (Scharf et al., 1993; Scharf and Alley, 1993).

Typical southeastern growers' practices generally include applying high insurance N rates to soft red winter wheat between Zadok's Growth Stage (GS) 25 and GS-30 (Zadoks et al., 1974). Some state environmental regulations are attempting to reduce these high inputs by basing N rates on wheat yield goals and basic soil properties such as texture and productivity (North Carolina Nutrient Management Workgroup, 2003). All of these approaches fail to take into consideration factors unique to a specific field and season, and consequently may often result in N rates being over- or under-applied.

Plant tissue tests have been successfully used to determine in-season N status of several grain crops (Hargrove et al., 1983; Donohue and Brann, 1984; Baethgen and Alley, 1989; Roth et al., 1989; Follett et al., 1992; Blackmer and Schepers, 1994; Smeal and Zhang, 1994). These in-season determinations of N status allow growers to optimize N rates by accounting for past growing conditions, residual soil N, and loss of N due to denitrification or leaching.

In wheat, whole-plant N concentration (g kg^{-1}) and wheat N uptake (kg N ha^{-1}) of above ground biomass have been the most widely studied in-season plant analyses for optimizing N recommendations. Several studies have reported GS-30 critical values (values above which a response to N fertilization is not expected) for whole-plant N concentration. Growth stage 30 is important because applying topdress N at this stage has been shown (Baethgen and Alley, 1989) to be the most efficient means of supplying N, and optimizing yield and N use efficiency (NUE). Critical whole-plant N concentration values of 35.0 g kg^{-1} (Roth et al., 1989), 36.0 g kg^{-1} (Fox et al., 1994), and 39.5 g kg^{-1} (Baethgen and Alley, 1989) have been reported. Critical GS-30 N uptake values of 48 kg N ha^{-1} (Roth et al., 1989) and 95 kg N ha^{-1} (Baethgen and Alley, 1989) have also been reported. These critical values for whole-plant N concentration or N uptake might be used to determine N sufficiency and aid in directing a N application.

While critical values are useful in identifying N-deficient crops, they do not allow growers to optimize N applications. Baethgen and Alley (1989) developed a linear relationship between GS-30 optimum N rate and both whole-plant N concentration and N uptake. Scharf et al. (1993) refined the linear relationship between GS-30 optimum N rate and whole-plant N concentration. These relationships provide a method to optimize N rates at the most critical time for achieving high NUE.

Plant tissue tests are expensive, difficult, and time consuming to obtain. Therefore, an inexpensive and quick way to determine in-season N status was sought. Several studies have reported that chlorophyll meter readings were related to whole-plant N concentration or grain yield (Follett et al., 1992; Reeves et al., 1993; Blackmer and Schepers, 1994; Fox et al., 1994; Murdock et al., 1997). Chlorophyll meters appeared to provide a quick and inexpensive method to determine in-season N status. However, studies also found that to accurately predict in-season N status and N rates across a wide range of environments a within-field calibration was required (Follett et al., 1992; Blackmer and Schepers, 1994; Fox et al., 1994; Smeal and Zhang, 1994). Typi-

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cally, within-field calibration uses the mean of several chlorophyll meter measurements in a non-N limited reference location. These non-N limited reference locations are achieved by applying a high (typically the maximum N rate used in that region) rate of N at an early GS (at planting or GS-25) to a small area in the field. Multiple chlorophyll meter measurements are obtained for these non-N limited reference locations and the mean is determined and used to adjust or calibrate subsequent in-field chlorophyll meter readings (Blackmer and Schepers, 1994; Fox et al., 1994; Smeal and Zhang, 1994; Murdock et al., 1997). A drawback to this within-field calibration technique is that each field must contain these non-N limited reference locations. Previous studies have also reported that GS-30 whole-plant N concentration and N uptake may vary spatially within a field (Roth et al., 1989). This spatial variability may be caused by many factors including but not limited to changes in soil properties, landscape position, and previous N applications. To account for this spatial variation, growers would need to use multiple reference areas and make intensive chlorophyll meter readings to obtain an accurate field average.

A solution to this problem may be the use of remote sensing in the form of an on-the-go sensor or aerial photograph. This may be possible because chlorophyll and N concentration influence reflectance in the blue (B), green (G), and red (R) regions of the visible spectrum (Gates et al., 1965; Knipling, 1970). Reflectance in the near-infrared (NIR) region of the spectrum (>700 nm) is influenced by vegetative cover and vigor (Knipling, 1970). Therefore, these wavelengths may be related to whole-plant N concentration or N uptake at GS-30 in wheat.

Previous research in wheat has shown that N applications alter the visible and NIR reflectance spectrum (Serrano et al., 2000). Using an on-the-go sensor, Sembiring et al. (2000) reported that wheat N uptake at GS-30 is correlated ($0.43 \leq R^2 \leq 0.67$) with the normalized difference vegetation index (NDVI). However, they reported that NDVI was not significantly correlated with whole-plant N concentration at GS-30. Lukina et al. (2001) also reported a strong relationship ($R^2 = 0.75$) between NDVI and wheat N uptake at GS-30. Stone et al. (1996) used an on-the-go sensor and found that the plant N spectral index (PNSI, the inverse of |NDVI|) was correlated with wheat N uptake at GS-30 ($0.41 \leq R^2 \leq 0.80$).

Remote sensing in the visible and NIR appears to have potential for determining within-field variability in crop N status. Consequently, our first objective was to determine if a spectral index or a single NIR, R, or G band derived from false color infrared aerial photographs could be used to estimate GS-30 whole-plant N concentration or N uptake in soft red winter wheat. Specifically, we wanted to determine if a remotely sensed, in-season estimate of N status could be used to predict optimum N rate using a previously derived linear relationship between N uptake (Baethgen and Alley, 1989) or whole-plant N concentration (Scharf et al., 1993) and optimum N rate. Because a within-field reference was required for determining N status with a chlorophyll meter, our second objective was to determine if the use of a non-N limited reference would improve the potential for using remote sensing to estimate GS-30 whole-plant N concentration or N uptake.

MATERIALS AND METHODS

Site Description

Research was conducted at eight sites in the coastal plain and piedmont regions of North Carolina in 1998, 1999, and 2000. In 1998, a site was located at the Tidewater Research Station (T-1) near Plymouth, NC (Fig. 1). In 1999, an on-farm site located near Wilson, NC (W-1), and a site on the Piedmont Research Station (P-1) located near Salisbury, NC, were used. In 2000, another Piedmont Research Station site (P-2) along with a site located on the Cunningham Research Station (K-1) and three sites on the Lower Coastal Plain Tobacco Research Station (K-2, K-3, and K-4) located near Kinston, NC, were used. Table 1 describes the soil types found at each site. At seven of these sites, GS-25 N rates were varied to produce different GS-30 whole-plant N concentrations and N uptake values (Table 2). Table 2 further describes the wheat varieties,

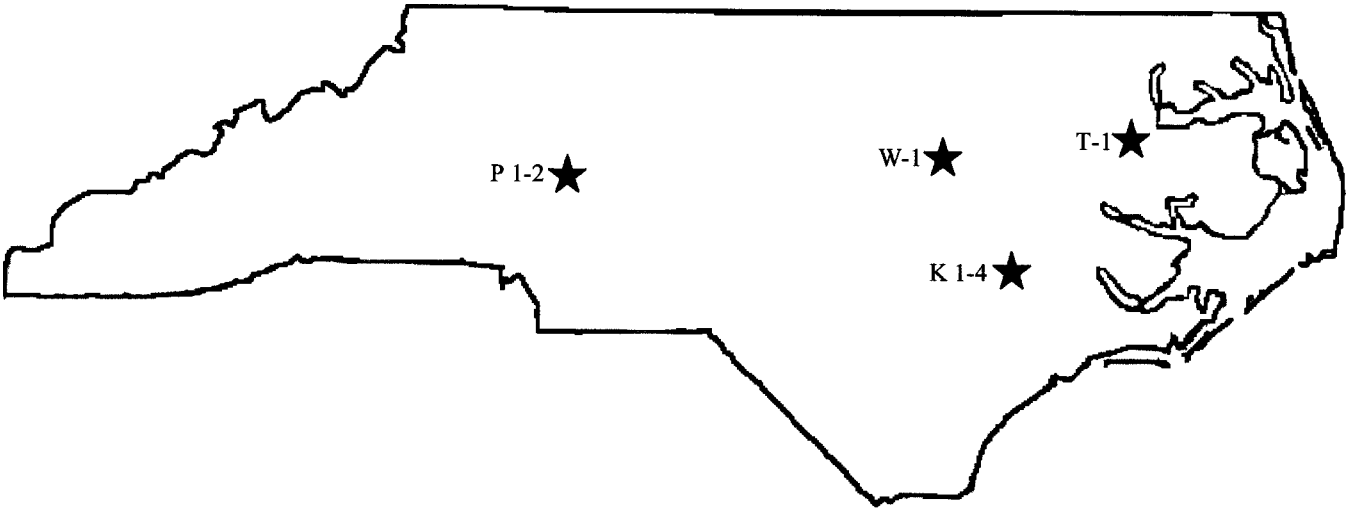

Fig. 1. Map of North Carolina. Site names are given adjacent to stars indicating site location.

Table 1. Soil type and soil taxonomic class at each of the eight site–years.

Site	Soil type	Soil taxonomic class
T-1	Portsmouth fine sandy loam	fine-loamy over sandy or sandy skeletal, mixed, thermic Umbric Paleaquults
P-1	Hiwassee clay loam	fine, kaolinitic, thermic Typic Rhodudults
W-1	Faceville sandy loam	clayey, kaolinitic, thermic Typic Kandiudults
P-2	Hiwassee clay loam	fine, kaolinitic, thermic Typic Rhodudults
K-1	Lynchburg sandy loam	fine, loamy, siliceous, thermic Aeric Paleaquults
K-2	Rains sandy loam	fine, loamy, siliceous, thermic Typic Paleaquults
K-3	Goldsboro loamy sand	fine, loamy, siliceous, thermic Aquic Paludults
K-4	Goldsboro loamy sand	fine, loamy, siliceous, thermic Aquic Paludults

planting date, row spacing, seeding rates, tillage system, GS-25 N rates, plot size, and number of plots at each site.

At T-1, a replicated strip plot factorial design with one variety, three seeding rates, and five GS-25 N rates was used. A randomized complete block design with six replications, three wheat varieties, three seeding rates, and four GS-25 N rates was used at P-1 (Table 2). All other sites received a single seeding rate and variety. The W-1 site received a single GS-25 N rate, while K-1, K-2, K-3, K-4, and P-2 received three GS-25 N rates. One sample location per plot was used for analysis at all sites. Ammonium nitrate (34–0–0) fertilizer was used for GS-25 N applications at T-1 and P-2, while liquid urea–ammonium nitrate (30–0–0) was used for GS-25 N applications at all other sites.

At all sites, a GS-30 plant tissue sample was collected at the center of each sample location by compositing two 1-m sections of row within a 1.2-m radius circle. The tissue sample consisted of all tissue above the soil surface. For each sample, dry biomass was determined by drying the sample at 60°C for 48 h. Whole-plant N concentration was determined using a CHN analyzer (McGeehan and Naylor, 1988). Nitrogen uptake at each sample location was determined by multiplying dry biomass by whole-plant N concentration.

Aerial Photography

Remote sensing was performed as described previously (Flowers et al., 2001). Latitude and longitude for all sample locations and of aerial targets placed at each field corner were determined using a differential global positioning systems (DGPS) receiver with 1-m accuracy (Trimble AG 132, Trimble Navigation, LTD, Sunnyvale, CA). Aerial photographs were taken from a belly mounted platform using a 35-mm Canon 81 camera (Cannon USA, Lake Success, NY) within 1 wk (5 Mar. 1999 at T-1, 15 Mar. 2000 at P-1 and W-1, and 2 Feb. 2001 at K-1, K-2, K-3, K-4, and P-2) of plant tissue sampling. Color infrared film (CIR, Kodak Ektachrome 153) along with a Kodak Wratten gelatin filter number 15 (Eastman Kodak Co., Rochester, NY) were used for the aerial photographs. A series of aerial photographs with differing exposures bracketed on F/8 and a shutter speed of 0.004 s (1/250 s) were taken at each site. All CIR film was AR-5 processed to obtain false CIR slides. All aerial photographs were taken as low as possible while ensuring that each site was contained in a single photograph. Aerial photographs were taken on cloudless days between 1200 and 1400 h Standard Time.

Digitization of Images and Photographic Analysis

Photographic analysis and digitization of images were performed on the positive false color slides from the CIR film as described by Flowers et al. (2001). Slides were digitized using the procedure described by Blackmer et al. (1996) with a Konica slide scanner (Konica Q-Scan, Konica Corp., Mahwah, NJ) and the software package Adobe Photoshop v 4.0 (Adobe Systems, San Jose, CA). The brightness and contrast were not adjusted on the digitized image. The digitized image was not sharpened using the scanner software. The image was scanned

Table 2. Wheat variety, planting date, row spacing, seeding rates, GS-25 N rates, tillage system, plot size, and total number of plots at each of the eight site–years.

Site	Wheat variety	Planting date	Row spacing	Seeding rates	GS-25 N rate	Tillage system	Plot size	No. of plots
			m	seeds m^{-2}	kg N ha^{-1}		m	
T-1	Coker ‘9663’	21 Nov. 1998	0.19	538	0	Conventional	12 by 1.5	87
				268	45			
				108	90			
					135			
					180			
P-1	Coker ‘9704’	19 Oct. 1999	0.19	640	0	No-till	3.1 by 3.1	180
	Pioneer ‘2580’			394	34			
	‘Roane’			180	67			
					101			
					135			
W-1	FFR ‘518’	26 Oct. 1999	0.19	414	67	No-till	15.4 by 15.4	71
P-2	Coker ‘9704’	24 Oct. 2000	0.19	394	0	No-till	9.2 by 4.6	72
					67			
					135			
K-1	Coker ‘9704’	20 Oct. 2000	0.15	467	0	Conventional	9.2 by 4.6	78
					67			
					135			
K-2	‘Roane’	23 Oct. 2000	0.15	467	0	Conventional	9.2 by 4.6	78
					67			
					135			
K-3	Pioneer ‘26R91’	3 Nov. 2000	0.15	467	0	Conventional	9.2 by 4.6	78
					67			
					135			
K-4	‘Roane’	23 Oct. 2000	0.15	467	0	Conventional	9.2 by 4.6	78
					67			
					135			

with a resolution of 47 pixels mm^{-1} with each pixel representing a range in ground area of 0.07 m^2 at T-1 to 0.42 m^2 at K-2. The range in ground area was due to differences in altitude when the image was taken. Each field was contained within a single aerial photograph, and consequently all comparisons were limited to within a given photograph. The digitized images were rectified in ERDAS Imagine (ERDAS, 1997) using the latitude and longitude of the aerial targets at each site. Root mean square error (RMSE) was calculated for each rectified image and ranged between 0.1 and 1.5 m.

Spectral Indices

Digital counts representing the spectral reflectance for each sample location were derived using ERDAS Imagine as described by Flowers et al. (2001). Color infrared film emulsions respond to light within the visible and NIR (490–900 nm) regions of the electromagnetic spectrum. The digitized images are represented by 24 bit true color with three bands [8 bit red (R), 8 bit green (G), and 8 bit blue (B)]. At each pixel in the image, the primary color value represents RGB digital counts within the range from 0 to 255. The spectral properties of CIR film result in wide overlapping wavelength bands. In our case band 1 (NIR) of the image covered the wavelengths between 490 and 900 nm, while band 2 (R) covered the wavelengths between 490 and 700 nm, and band 3 (G) covered the wavelengths between 490 and 620 nm (Eastman Kodak Co., Rochester, NY). While these bands overlap, differences in spectral sensitivity exist between them. Maximum sensitivity in the NIR band occurs at 730 nm, in the R band at 650 nm, and in the G band at 550 nm. These differences in spectral sensitivity may offer increased information through the use of a spectral index.

In that light, several spectral indices in addition to digital counts for each band (NIR, R, and G) were examined. An NDVI (Yang and Anderson, 1999) was determined using the digital counts from the NIR and R bands such that:

$$\text{NDVI} = (\text{NIR} - \text{R})/(\text{NIR} + \text{R}) \quad [1]$$

A green normalized vegetation difference index (GNDVI) (Gitelson et al., 1996) was calculated using the NIR and G bands as:

$$\text{GNDVI} = (\text{NIR} - \text{G})/(\text{NIR} + \text{G}) \quad [2]$$

A normalized NIR value (Jain, 1989) was derived from all bands such that:

$$\text{Normalized NIR} = \text{NIR}/(\text{NIR} + \text{R} + \text{G}) \quad [3]$$

A ratio vegetation index (RVI) (Jordan, 1969) and a difference vegetation index (DVI) (Tucker, 1979) were calculated as:

$$\text{RVI} = \text{NIR}/\text{R} \quad [4]$$

$$\text{DVI} = \text{NIR} - \text{R} \quad [5]$$

Data Analysis

Regression analysis (general linear models, SAS Inst., 1998) was used to determine if a significant relationship (linear or quadratic) existed between GS-30 whole-plant N concentration or GS-30 N uptake and NIR, R, and G digital counts, NDVI, GNDVI, Normalized NIR, RVI, and DVI at all sites.

Stone et al. (1996) reported that PNSI was correlated (R^2 = 0.61) with GS-30 N uptake across sites. Therefore, regression analysis (general linear models, SAS Inst., 1998) of the relationship between GS-30 whole-plant N concentration and GS-30 N uptake and NDVI (linear, quadratic, and exponential models) was examined across sites.

Blackmer and Schepers (1994) reported a within-field calibration technique for monitoring N status in corn (*Zea mays* L.) with a chlorophyll meter. This technique was modified to calculate a NDVI sufficiency N index using a high (135 kg N ha^{-1} GS-25 N application) reference as:

$$\text{NDVI sufficiency index} = \text{NDVI}_{\text{SL}}/\text{NDVI}_{\text{135 Mean}} \quad [6]$$

where NDVI_{SL} is the NDVI value for the sample location and $\text{NDVI}_{\text{135 Mean}}$ is the mean NDVI value for the 135 kg N ha^{-1} plots. Regression analysis (general linear models, SAS Inst., 1998) was used to determine if a significant relationship (linear, quadratic, or exponential) existed between GS-30 whole-plant N concentration or N uptake and NDVI sufficiency index across sites.

Our final objective was to determine if previously published relationships between GS-30 optimum N rate and either whole-plant N concentration (Scharf et al., 1993) or N uptake (Baethgen and Alley, 1989) could be used to develop an empirical relationship between NDVI and optimum N rate. Scharf et al. (1993) reported a relationship between GS-30 optimum N rate and GS-30 whole-plant N concentration such that:

$$\text{PredNRate}_{\text{NConcentrationBased}}\ (\text{kg N ha}^{-1}) = 235 - 4.8 \times \text{GS-30 Whole-Plant N Conc. (g kg}^{-1}) \quad [7]$$

where $\text{PredNRate}_{\text{NConcentrationBased}}$ is the GS-30 optimum N rate. Equation [7] was used to substitute $\text{PredNRate}_{\text{NConcentrationBased}}$ for GS-30 whole plant N concentration in the relationship between NDVI and GS-30 whole-plant N concentration found in our experiments (see Results and Discussion section, Fig. 3 and 7).

Baethgen and Alley (1989) reported a relationship between GS-30 optimum N rate and N uptake such that:

$$\text{PredNRate}_{\text{NUptakeBased}}\ (\text{kg N ha}^{-1}) = 237 - 2.17 \times \text{GS-30 N Uptake (kg N ha}^{-1}) \quad [8]$$

where $\text{PredNRate}_{\text{NUptakeBased}}$ is the GS-30 optimum N rate. Equation [8] was used to substitute $\text{PredNRate}_{\text{NUptakeBased}}$ for GS-30 N uptake in the relationship between NDVI and N uptake found in our experiments (see Results and Discussion section, Fig. 4 and 8). Regression analysis (general linear models, SAS Inst., 1998) was used to determine if a significant relationship (linear, quadratic, or exponential) existed between NDVI and $\text{PredNRate}_{\text{NConcentrationBased}}$ or $\text{PredN Rate}_{\text{NUptakeBased}}$ across sites.

RESULTS AND DISCUSSION

GS-30 Whole-Plant Nitrogen Concentration

Whole-plant GS-30 N concentration was significantly related to several spectral indices and individual bands at all sites (Table 3). However, coefficients of determination (R^2) were variable, ranging from 0.04 to 0.72. Clarke et al. (2000, 2001) reported that biomass or canopy density influenced the relationship between spectral reflectance and chlorophyll concentration, which is related to whole-plant N concentration. Mean GS-30 biomass was 765, 1039, 525, 1431, 1030, 717, 1059, and 350 kg ha^{-1} at T-1, P-1, W-1, K-1, K-2, K-3, K-4, and P-2, respectively. Consistent with the findings of Clarke et al. (2000, 2001), sites that had a relatively strong ($R^2 >$ 0.40) relationship (Table 3) between GS-30 whole-plant N concentration and either a spectral index or individual

Table 3. Regression analysis of whole-plant GS-30 N concentration (N, g kg^{-1}) with near infrared (NIR), red (R), green (G), normalized difference vegetation index (NDVI), green normalized difference vegetation index (GNDVI), normalized NIR, ratio vegetation index (RVI), and difference vegetation index (DVI) for eight site–years. The model significance (Sig) and the coefficient of determination (R^2) for both the linear and quadratic models are given.

		Spectral index															
		NIR		R		G		NDVI		GNDVI		Normalized NIR		RVI		DVI	
Site	Source of variation	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2
T-1	N (linear)	NS	–	*	0.17	*	0.11	*	0.08	NS	–	NS	–	NS	–	*	0.10
	N (quadratic)	NS		*		*		*		NS		NS		NS		*	
P-1†	N (linear)	*	0.37	*	0.68	*	0.68	*	0.72	*	0.66	*	0.69	*	0.68	*	0.71
	N (quadratic)	*		*		*		*		*		*		*		*	
P-1‡	N (linear)	*	0.21	*	0.62	*	0.63	*	0.65	*	0.62	*	0.64	*	0.62	*	0.61
	N (quadratic)	*		*		*		*		*		*		*		*	
P-1§	N (linear)	*	0.34	*	0.51	*	0.54	*	0.51	*	0.52	*	0.53	*	0.48	*	0.45
	N (quadratic)	*		*		*		*		**		*		NS		*	
W-1	N (linear)	*	0.11	*	0.19	*	0.16	*	0.14	*	0.12	*	0.13	*	0.11	*	0.19
	N (quadratic)	*		*		*		*		*		*		*		*	
K-1	N (linear)	*	0.19	*	0.61	*	0.54	*	0.62	*	0.53	*	0.58	*	0.60	**	0.59
	N (quadratic)	*		*		*		**		NS		NS		**		NS	
K-2	N (linear)	*	0.22	*	0.43	*	0.55	*	0.44	*	0.40	*	0.46	*	0.47	*	0.40
	N (quadratic)	NS		NS		NS		NS		NS		NS		NS		NS	
K-3	N (linear)	NS	–	*	0.21	*	0.21	*	0.17	*	0.12	*	0.15	*	0.17	*	0.16
	N (quadratic)	NS		NS		NS		NS		NS		NS		NS		NS	
K-4	N (linear)	*	0.47	*	0.48	*	0.55	*	0.48	*	0.45	*	0.48	*	0.50	*	0.44
	N (quadratic)	NS		NS		NS		NS		NS		NS		NS		NS	
P-2	N (linear)	**	0.04	*	0.26	*	0.29	*	0.30	*	0.33	*	0.31	*	0.28	*	0.30
	N (quadratic)	NS		*		*		*		*		*		*		*	

*** Indicates model was significant at the 5% level.**
**** Indicates model was significant at the 10% level.**
† Coker '9704' wheat variety at P-1.
‡ Pioneer '2580' wheat variety at P-1.
§ Roane wheat varieties at P-1.

band (P-1, K-1, K-2, and K-4) also had high (>1000 kg ha^{-1}) mean GS-30 biomass (Fig. 2). Conversely, sites that had a poor or nonsignificant relationship between GS-30 whole-plant N concentration and either a spectral index or individual band (T-1, W-1, K-3, and P-2) had low (<1000 kg ha^{-1}) mean GS-30 biomass.

Several factors that may influence GS-30 biomass include planting date, seeding rate, and environmental factors such as rainfall and temperature. In our study, seeding rates were varied at two sites (T-1 and P-1) to achieve a range in GS-30 biomass. At P-1, environmental conditions were excellent for early wheat growth and all seeding rates resulted in high (>1000 kg ha^{-1}) GS-30 biomass. Conversely, at T-1 cold weather slowed wheat growth and all seeding rates resulted in low (<1000 kg ha^{-1}) GS-30 biomass. As a result, environmental conditions were more important than seeding rates in determining GS-30 biomass at these sites and the seeding rates did not have a significant influence on the relationship between GS-30 whole-plant N concentration and a spectral index or individual band.

It was possible to pool across high mean GS-30 biomass sites and develop a single relationship between GS-30 whole-plant N concentration and either a spectral

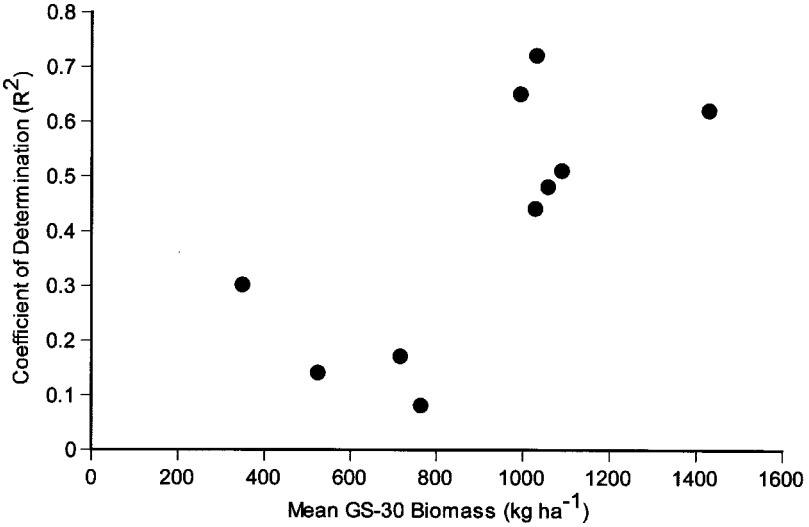

Fig. 2. The coefficient of determination (R^2) for the linear or quadratic model between growth stage (GS)-30 whole-plant N concentration and the normalized difference vegetation index (NDVI) vs. mean GS-30 biomass.

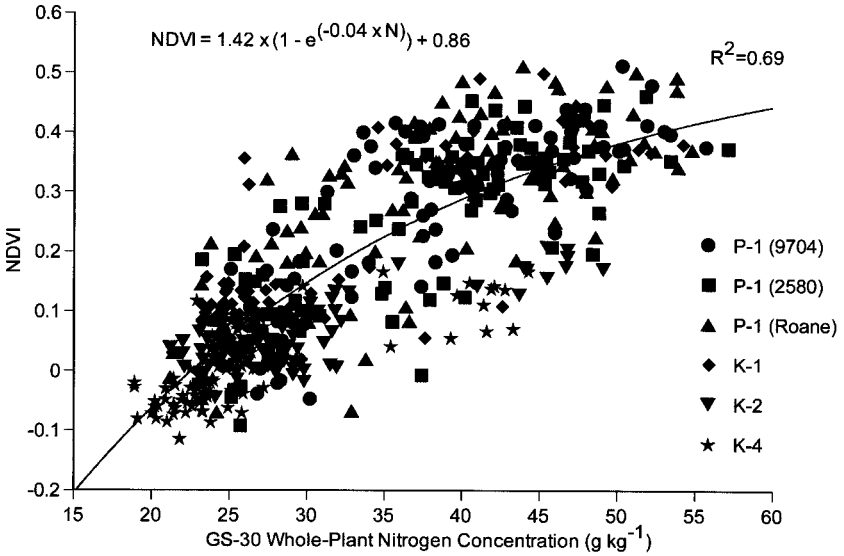

Fig. 3. The normalized difference vegetation index (NDVI) vs. growth stage (GS)-30 whole-plant N concentration for the four site–years with high (>1000 kg ha^{-1}) mean GS-30 biomass.

index or band. Previous studies (Stone et al., 1996; Sembiring et al., 2000) used NDVI or PNSI to estimate crop N status. We also found NDVI to be among the best estimators of GS-30 whole-plant N concentration at high mean GS-30 biomass sites (Table 3). Figure 3 shows the relationship between GS-30 whole-plant N concentration and NDVI. This exponential relationship was found to account for 69% of the variation between GS-30 whole-plant N concentration and NDVI pooled across high mean GS-30 biomass sites.

GS-30 Nitrogen Uptake

All spectral bands and indices except NIR at T-1 were significantly related with GS-30 N uptake (Table 4). Similar to the findings of Stone et al. (1996), Sembiring et al. (2000), and Lukina et al. (2001), NDVI was among the best estimators of GS-30 N uptake at all sites (Table 4).

Three sites (K-2, K-3, and K-4) were infected with Hessian fly [*Mayetiola destructor* (Say)]. Hessian fly larvae feed on the developing lower whorl and can cause

Table 4. Regression analysis of GS-30 N uptake (Nup) with near infrared (NIR), red (R), green (G), normalized difference vegetation index (NDVI), green normalized difference vegetation index (GNDVI), normalized NIR, ratio vegetation index (RVI), and difference vegetation index (DVI) for eight site–years. The model significance (Sig) and the coefficient of determination (R^2) for both the linear and quadratic models are given.

		Spectral index															
		NIR		R		G		NDVI		GNDVI		Normalized NIR		RVI		DVI	
Site	Source of variation	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2	Sig	R^2
T-1	Nup (linear)	NS	–	*	0.52	*	0.50	*	0.54	*	0.50	*	0.53	*	0.53	*	0.51
	Nup (quadratic)	NS		*		*		*		*		*		*		*	
P-1†	Nup (linear)	*	0.41	*	0.71	*	0.72	*	0.75	*	0.73	*	0.75	*	0.73	*	0.71
	Nup (quadratic)	*		*		*		*		*		*		*		*	
P-1‡	Nup (linear)	*	0.21	*	0.70	*	0.71	*	0.75	*	0.74	*	0.76	*	0.72	*	0.70
	Nup (quadratic)	*		*		*		*		*		*		*		*	
P-1§	Nup (linear)	*	0.48	*	0.75	*	0.76	*	0.70	*	0.68	*	0.69	*	0.61	*	0.67
	Nup (quadratic)	*		*		*		*		*		*		*		*	
W-1	Nup (linear)	*	0.09	*	0.48	*	0.36	*	0.53	*	0.42	*	0.49	*	0.46	*	0.60
	Nup (quadratic)	NS		*		**		*		**		*		NS		*	
K-1	Nup (linear)	*	0.15	*	0.59	*	0.53	*	0.60	*	0.53	*	0.58	*	0.58	*	0.58
	Nup (quadratic)	*		*		*		*		*		*		*		*	
K-2	Nup (linear)	*	0.21	*	0.36	*	0.39	*	0.36	*	0.27	*	0.35	*	0.36	*	0.34
	Nup (quadratic)	NS		*		*		*		*		*		*		*	
K-3	Nup (linear)	*	0.14	*	0.48	*	0.46	*	0.49	*	0.46	*	0.48	*	0.49	*	0.49
	Nup (quadratic)	NS		NS		NS		NS		NS		NS		NS		NS	
K-4	Nup (linear)	*	0.41	*	0.49	*	0.56	*	0.51	*	0.52	*	0.53	*	0.52	*	0.47
	Nup (quadratic)	NS		NS		NS		NS		NS		NS		NS		NS	
P-2	Nup (linear)	*	0.16	*	0.39	*	0.47	*	0.48	*	0.59	*	0.53	*	0.47	*	0.48
	Nup (quadratic)	NS		NS		NS		NS		NS		NS		NS		NS	

* Indicates model was significant at the 5% level.
** Indicates model was significant at the 10% level.
† Coker '9704' at P-1.
‡ Pioneer '2580' at P-1.
§ Roane wheat varieties at P-1.

tiller stunting or death (Van Duyn et al., 2000). Since GS-30 N uptake is related to biomass, the relationship between GS-30 N uptake and NDVI would likely have been altered due to Hessian fly damage at these sites. In fact, at these three sites, NDVI at low GS-30 N uptake values (40–70 kg N ha^{-1}) were lower than found at other sites (data not shown). Previous studies have also shown that insect damage is detectable using remote sensing (Wildman, 1982). Therefore, these sites were not included in further analyses to minimize extraneous interferences.

Figure 4 shows the relationship between GS-30 N uptake and NDVI at all sites without Hessian fly. This exponential relationship accounted for 61% of the variation between GS-30 N uptake and NDVI and is very similar to the relationship reported by Lukina et al. (2001). An important difference between our relationship (Fig. 4) and the relationship reported by Lukina et al. (2001) is that in our relationship high GS-30 N uptake values (>100 kg N ha^{-1}) saturate NDVI. Saturation is a common characteristic in the relationships between NDVI and plant properties. Serrano et al. (2000) reported that the relationship between NDVI and leaf area index multiplied by chlorophyll *a* concentration (similar to N uptake) saturated at values >1000 mg m^{-2}. Similarly, Gitelson et al. (1996) reported that the relationship between NDVI and chlorophyll *a* concentration saturated at concentrations >20 μg cm^{-2}. Saturation of the relationship between NDVI and GS-30 N uptake makes the differentiation of NDVI values at high GS-30 N uptake values difficult. This may limit the usefulness of using NDVI to predict GS-30 N uptake.

NDVI Sufficiency Index

Blackmer and Schepers (1994) reported that the use of a sufficiency index based on a non-N limited reference area improved the relationship between relative grain yield and chlorophyll meter readings. They also reported a strong relationship between the sufficiency index and whole-plant N concentration. The sufficiency index is based on an underlying assumption that spectral measurements are related to GS-30 whole-plant N concentration. Sites with low mean GS-30 biomass did not have a strong relationship with GS-30 whole-plant N concentration (Table 3, Fig. 2), and thus violate this assumption. Therefore, only the four sites (P-1, K-1, K-2, and K-4) with mean GS-30 biomass >1000 kg ha^{-1} and reference locations within the field that received a high N rate (135 kg N ha^{-1}) were examined.

An NDVI sufficiency index was calculated (Eq. [6]) for each sample location. Figure 5 shows the relationship (exponential, $R^2 = 0.66$) between GS-30 whole-plant N concentration and the NDVI sufficiency index at high mean GS-30 biomass sites. While this resulted in a strong relationship, there was not a substantial improvement in the estimation of whole plant N concentration using the NDVI sufficiency index (Eq. [6]) compared with the direct measurement of NDVI (Fig. 3).

The relationship between GS-30 N uptake and a NDVI sufficiency index was also examined. Three sites (K-2, K-3, and K-4) were removed to eliminate effects due to Hessian fly damage and one site was removed due to lack of an in-field high (135 kg N ha^{-1}) reference location. The relationship between the NDVI sufficiency index and GS-30 N uptake varied by site–year (Fig. 6). At T-1 and P-2 the NDVI sufficiency index values are higher at low GS-30 N uptake values (20–40 kg N ha^{-1}) compared with P-1 (Fig. 6). At medium and high GS-30 N uptake values (60–140 kg N ha^{-1}) the NDVI sufficiency index values are higher for K-1 compared with P-1. At P-1, the NDVI sufficiency index appears to follow a curvilinear relationship similar to the exponential relationship between NDVI and GS-30 N uptake. Due to these differences between sites, there

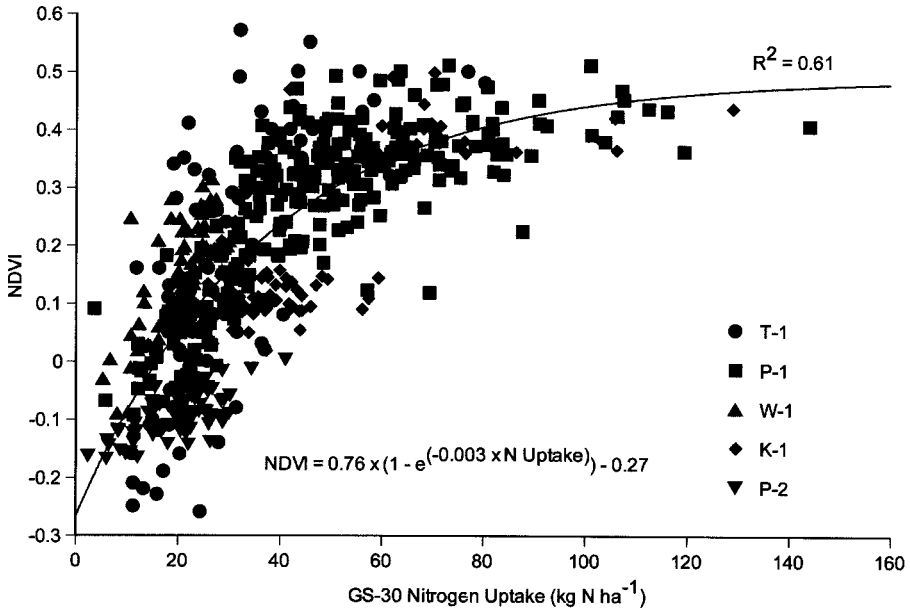

Fig. 4. The normalized difference vegetation index (NDVI) vs. growth stage (GS)-30 N uptake for five site–years without Hessian fly infestations. Note P-1 was not separated by variety.

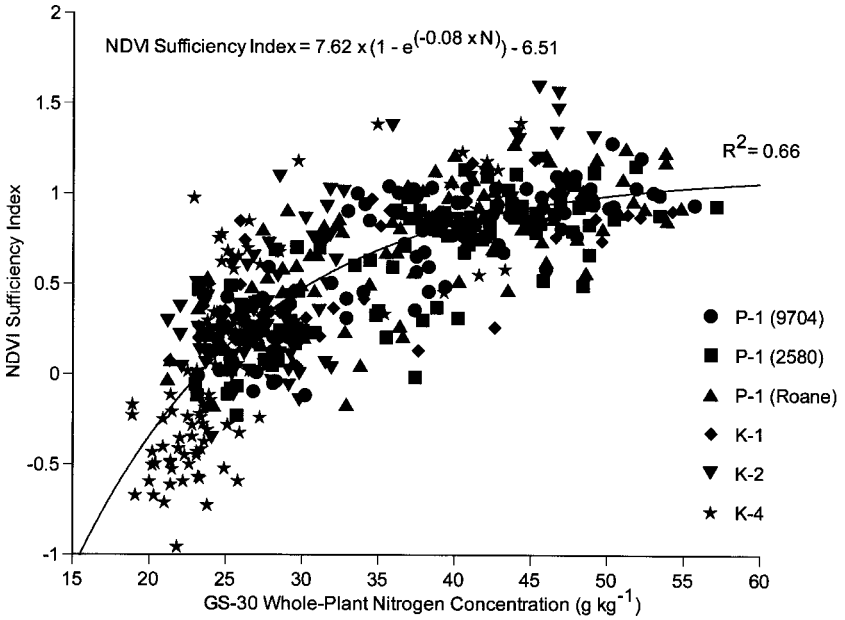

Fig. 5. The normalized difference vegetation index (NDVI) sufficiency index vs. growth stage (GS)-30 whole-plant N concentration for four site–years with high (>1000 kg ha^{-1}) GS-30 mean biomass.

was not an improvement in estimating GS-30 N uptake using the NDVI sufficiency index (Fig. 6) compared with the direct measurement of NDVI (Fig. 4).

Predicted Nitrogen Rate Based on Spectral Indices

Converting GS-30 whole-plant N concentration values in Fig. 3 to PredNRate$_{\text{NConcentrationBased}}$ based on Eq. [7], an empirical relationship between NDVI and PredNRate$_{\text{NConcentrationBased}}$ was developed at sites with high mean GS-30 biomass (Fig. 7). The R^2 for this relationship was 0.64, indicating that there is a possibility of using NDVI to estimate PredNRate$_{\text{NConcentrationBased}}$ for soft red winter wheat. It should be noted that Eq. [7], while based on 32 site–years of data (Scharf et al., 1993), had an R^2 of 0.51. Since Fig. 7 uses this relationship the actual R^2 between NDVI and PredNRate$_{\text{NConcentrationBased}}$ could be significantly higher or lower than 0.64 reported here.

Calculating PredNRate$_{\text{NUptakeBased}}$ based on GS-30 N uptake in Fig. 4 using Eq. [8], an empirical relationship between NDVI and PredNRate$_{\text{NUptakeBased}}$ was developed (Fig. 8). Figure 8, however, illustrates two critical problems in using NDVI to estimate PredNRate$_{\text{NUptakeBased}}$ as

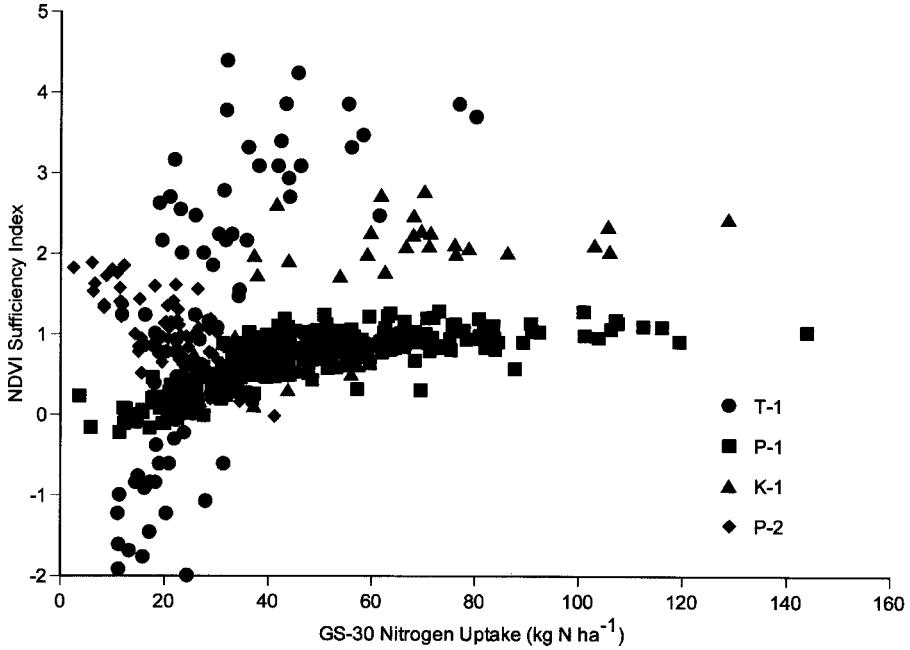

Fig. 6. The normalized difference vegetation index (NDVI) sufficiency index vs. growth stage (GS)-30 N uptake for four site–years with high (135 kg N ha^{-1}) reference locations and without Hessian fly infestations.

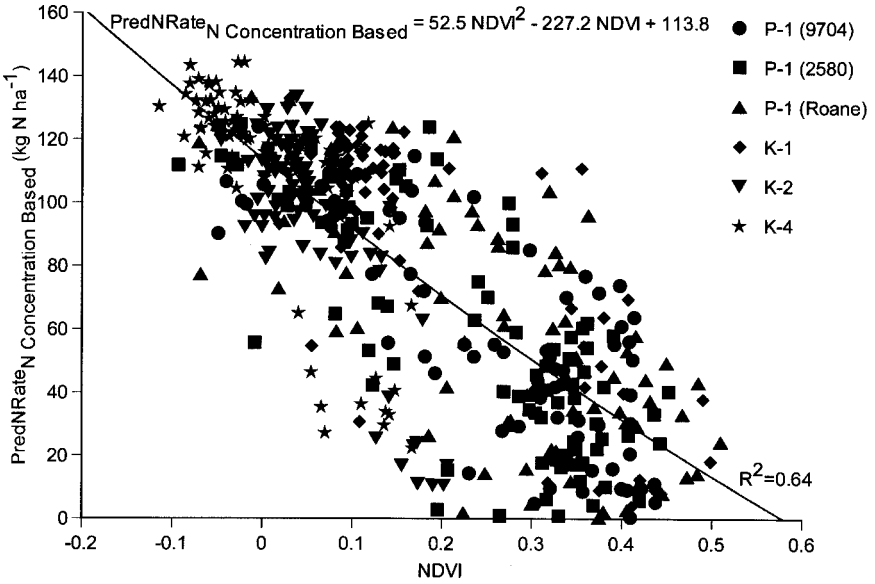

Fig. 7. Relationship between $PredNRate_{NConcentrationBased}$ vs. the normalized difference vegetation index (NDVI) for four site–years with high (>1000 kg ha^{-1}) growth stage (GS)-30 biomass. Equation [7] was used to calculate $PredNRate_{NConcentrationBased}$ based on GS-30 whole-plant N concentration.

a function of GS-30 N uptake. First, while the R^2 reported in Fig. 8 is 0.57, the regression model does not accurately capture the saturation of NDVI values at low $PredNRate_{NUptakeBased}$ values. This contrasts to Fig. 4, which shows the saturation of NDVI at high GS-30 N uptake values. Thus, Fig. 8 is misleading in that it appears that NDVI may be used to differentiate between low $PredNRate_{NUptakeBased}$ values, when in fact the differentiation of low $PredNRate_{NUptakeBased}$ values is very poor. This severely limits the use of NDVI to predict $PredNRate_{NUptakeBased}$ (Fig. 8) based on N uptake.

The second problem Fig. 8 illustrated was that N rates up to 232 kg N ha^{-1} were recommended, which is nearly two times greater than wheat in this region could tolerate without serious yield reduction, disease infestations, and lodging (Weisz and Heiniger, 2000). Baethgen and Alley (1989) reported the sufficiency level for N uptake to be 95 kg N ha^{-1}. For N uptake values at or above this level, wheat yields did not respond to N fertilization. For N uptake values below sufficiency, N rates increased linearly. Roth et al. (1989) reported a much lower N uptake sufficiency level of 48 kg N ha^{-1} and demonstrated that it varied by site–year. This suggests that the relationship between N uptake and GS-30 optimum N rate is not consistent across sites or years, and therefore, limits using NDVI to predict $PredNRate_{NUptakeBased}$ (Fig. 8) as a function of GS-30 N uptake.

Figure 9 illustrates a third and even more serious problem with using GS-30 N uptake to estimate $PredNRate_{NUptakeBased}$. At every site–year, and across the entire

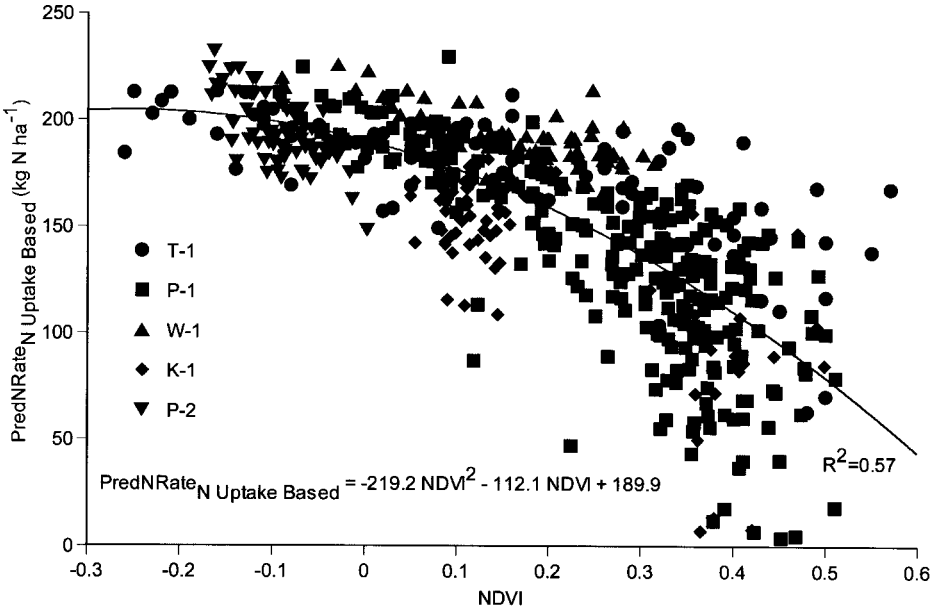

Fig. 8. Relationship between $PredNRate_{NUptakeBased}$ vs. the normalized difference vegetation index (NDVI) for five site–years. Equation [8] was used to calculate $PredNRate_{NUptakeBased}$ based on growth stage (GS) 30 N uptake. Note P-1 was not separated by variety.

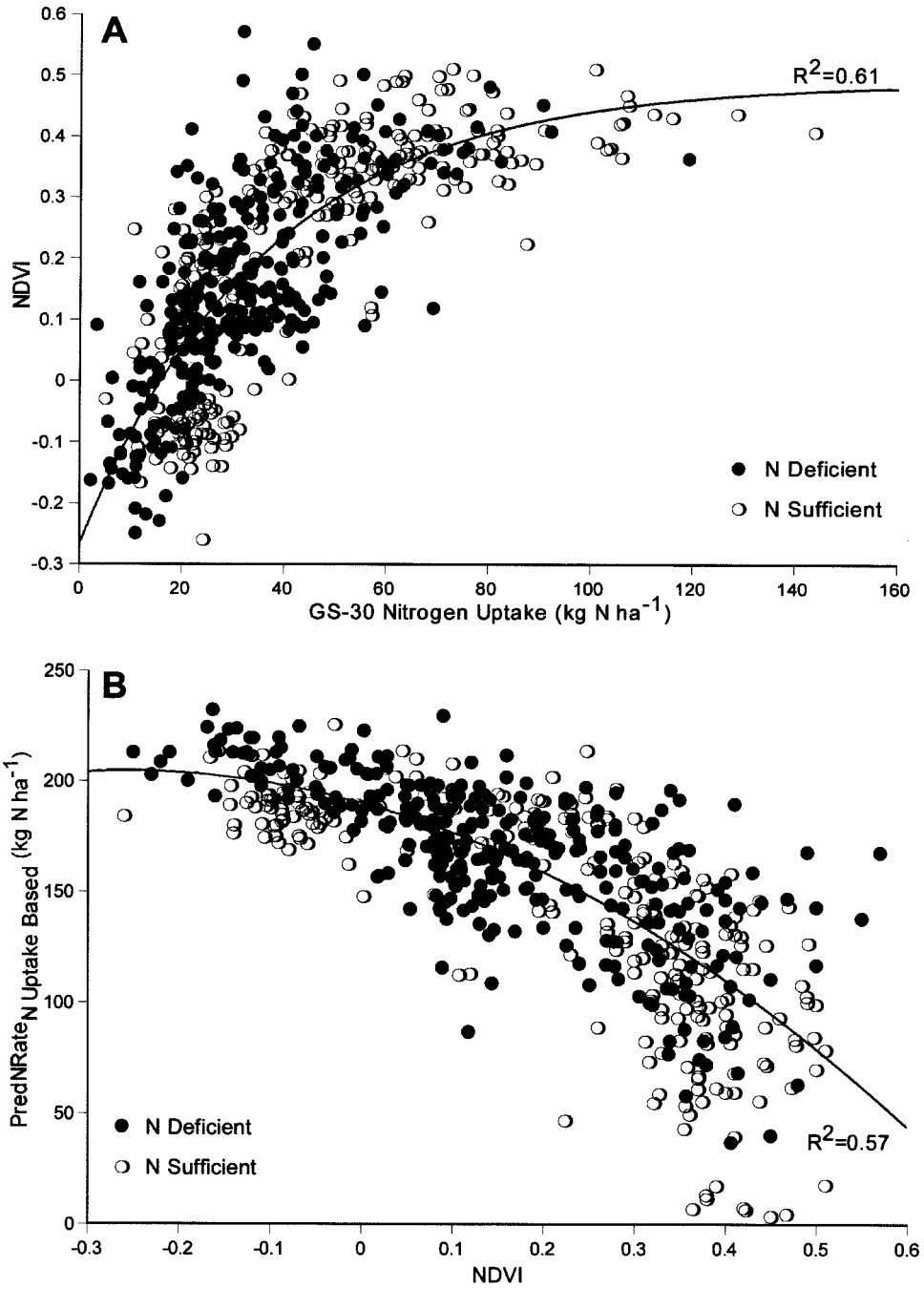

Fig. 9. Failure of growth stage (GS)-30 N uptake to distinguish between N deficient and N sufficient sample locations. Data are identical to those shown in Fig. 5 and 8; however, filled symbols represent sample locations with N deficiency based on the critical value of 39.5 g kg^{-1} for GS-30 whole-plant N concentration (Scharf et al., 1993). Open symbols represent sample locations with sufficient N also based on 39.5 g kg^{-1} for GS-30 whole-plant N concentration. (*A*) NDVI vs. GS-30 N uptake; (*B*) PredNRate$_{NUptakeBased}$ calculated from GS-30 N uptake (Eq. [8]) vs. NDVI.

range of N uptake values, there were individual sample locations that had sufficient N (Fig. 9A) based on the GS-30 whole-plant N concentration sufficiency value of 39.5 g kg^{-1} (Scharf et al., 1993). Unlike N uptake sufficiency values that are unstable across site–years, sufficiency values based on whole-plant N concentration have been shown to be highly stable across site–years and studies (Roth et al., 1989; Scharf et al., 1993; Fox et al., 1994). Consequently, each of these locations (Fig. 9A, *open symbols*), while being spread across the entire range of N uptake values, actually had a GS-30 optimum N rate of 0 kg N ha^{-1}. When NDVI and Eq. [8] were used to convert these N uptake values to PredNRate$_{NUptakeBased}$, N rates from 0 to >200 kg N ha^{-1} would have been recommended (Fig. 9B). Clearly, while NDVI can be used to estimate GS-30 N uptake (Fig. 4), NDVI cannot be used to determine PredNRate$_{NUptakeBased}$ as a function of N uptake.

CONCLUSIONS

Our first objective was to determine if a spectral index or a single NIR, R, or G band derived from false color infrared aerial photographs could be used to estimate whole-plant N concentration or N uptake at GS-30. Consistent with previous studies (Clarke et al., 2000, 2001), biomass was shown to influence the relationship between GS-30 whole-plant N concentration and spectral reflectance (Table 3, Fig. 2). This may explain why Sembiring et al. (2000) failed to find a consistent relationship between NDVI and GS-30 whole-plant N concentration. A strong ($R^2 = 0.69$) exponential relationship was found between NDVI and GS-30 whole-plant N concentration at high mean GS-30 biomass sites (Fig. 3). These data (Table 3, Fig. 2) make it clear that NDVI can only be used to estimate GS-30 whole-plant N concentration where GS-30 biomass is adequate (in our study >1000 kg ha^{-1}). Similar to Lukina et al. (2001), an exponential relationship ($R^2 = 0.61$) between NDVI and GS-30 N uptake was found (Fig. 4).

Our second objective was to determine if the use of a non-N limited reference would improve the relationship between a spectral index or a single NIR, R, or G band derived from false color infrared aerial photographs and whole-plant N concentration or N uptake at GS-30. Consistent with the results of Blackmer and Schepers (1994), a strong relationship (exponential, $R^2 = 0.66$) was found between the NDVI sufficiency index and GS-30 whole-plant N concentration (Fig. 5). However, the use of a non-N limited reference did not improve the relationship between NDVI (Eq. [6], NDVI sufficiency index) and GS-30 whole-plant N concentration (Fig. 5) compared with a direct estimate of GS-30 whole-plant N concentration by NDVI (Fig. 3).

Similar results were found for the relationship between the NDVI sufficiency index and GS-30 N uptake (Fig. 6). The use of a non-N limited reference did not improve the relationship between NDVI (Eq. [6], NDVI sufficiency index) and GS-30 N uptake compared with a direct estimate of GS-30 N uptake by NDVI (Fig. 4). This was due to differences in the NDVI sufficiency index values between GS-30 N uptake values of 20 to 40 kg N ha^{-1} at low and high mean GS-30 biomass sites.

Lastly, we wanted to determine if a spectral measurement could be used to predict GS-30 optimum N rate using the previously published relationships between GS-30 whole-plant N concentration (Scharf et al., 1993) or GS-30 N uptake (Baethgen and Alley, 1989). Figure 7 shows that empirically, it is possible to use NDVI to estimate $\text{PredNRate}_{\text{NConcentrationBased}}$ at sites with high biomass (>1000 kg ha^{-1}). However, because this relationship is based on two empirical relationships (Eq. [7] and Fig. 3), studies should be done to develop the direct relationship between GS-30 optimum N rate and NDVI.

The relationship between GS-30 N uptake and GS-30 optimum N rate is not stable across site–years (Roth et al., 1989) and resulted in unrealistically high $\text{PredNRate}_{\text{NUptakeBased}}$ estimates for N sufficient sample locations (Fig. 9) when using NDVI. Due to these serious problems, care should be taken when attempting to use NDVI to predict GS-30 optimum N rates as a function of N uptake.

Perhaps our most encouraging finding was the relationship between NDVI and whole-plant N concentration for high biomass sites. Because NDVI can be related to N concentration, and N concentration is related to optimum N rate for soft red winter wheat in this region, it should be possible to find a direct relationship between NDVI and optimum N rate. This should be a priority for future research in this area. Clarke et al. (2000 and 2001) reported promising results when using a remote sensing based two-dimensional approach to N management that first estimated crop biomass. Other studies have also shown promise for remote sensing based optimum N rates when accounting for crop biomass (Scharf et al., 2001). It may be that different relationships between optimum GS-30 N rates and NDVI or some other spectral measures will need to be developed for soft red winter wheat stands with different GS-30 biomass.

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